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SHORT COMMUNICATION

Tricalcium silicate induces enamel remineralization in human saliva

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Abstract *Background/purpose:* Severe tooth demineralization by production of acid leads to dental caries. In this study, we investigate the repairing effect of tricalcium silicate (Ca_3SiO_4) ceramics on acid-etched enamel in natural human saliva.
Materials and methods: The demineralization of enamel discs was simulated with citric acid, and they were then brushed with Ca_3SiO_4 paste. The treated discs were placed in human saliva for 1 day or 3 days at 36.5°C, and then the surface morphologies and structures were observed by atomic force microscopy (AFM) and transmission electron microscopy (TEM).
Results: Ca_3SiO_4 ceramics can induce enamel remineralization in human saliva and form a compact Ca—P mineralized layer onto the etched enamel surface. After soaking for 1 day, the thickness was 200—250 nm and the interface bonded well. The chemical components and structure of the mineralized layer were similar to those of the enamel matrix. After soaking for 3 days, the surface roughness of the enamel surface became lower.
Conclusion: The bioactive Ca_3SiO_4 repaired the acid-etched enamel successfully and protected the demineralized teeth.

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Introduction

Teeth withstand a range of physical and chemical processes in the oral cavity environment. In particular, the chemical attack coming from acidic foods or products of bacterial metabolism leads to enamel demineralization.[1] Being the hardest tissue of the body, mature enamel is acellular, having more than 96% mineral content and does not remodel once demineralized. Therefore, a synthetic remineralization agent is the only feasible way to repair the damage. Calcium silicate-based biomaterials, such as β-$CaSiO_3$, β-Ca_2SiO_4, and Ca_3SiO_4, play an important role in hard tissue reparative regeneration,[2] show good bioactivity and biocompatibility, and can induce bone-like apatite formation in simulated body fluid.[3] In particular, Ca_3SiO_4, a major component of Portland cement, is the optimal choice for repair of demineralized enamel as a remineralizing agent because it hydrates and adheres to the enamel surface very easily. Moreover, previous studies have demonstrated that Ca_3SiO_4 as a dental repair material showed good bioactivity and did not affect the specific functions of pulpal fibroblasts; it can be safely used in the clinic as a single bulk restorative material without any conditioning treatment.[4] Our previous study has also verified that Ca_3SiO_4 showed good remineralization potential in simulated oral fluid (SOF).[5] Owing to the complicated components of natural human saliva, we undertook further study on enamel remineralization with Ca_3SiO_4.

Materials and methods

Preparation and treatment of enamel samples

All the materials used in the paper were come from the Chinese medicine group in Shanghai, China. Freshly extracted human molars were cleaned and sectioned at the cemento-enamel junction using a precision slow speed diamond saw to obtain 3 mm $\times$ 3 mm $\times$ 1 mm enamel slabs. Samples were first polished with silicon carbide paper, and second with a polishing cloth with the slurries of 0.3 micro polishing compounds; they were then cleaned by ultrasound in an ethanol solution and further rinsed with distilled water before use.

Ca_3SiO_4 powders were prepared by the sol-gel method according to our previous study and sieved through a 300-mesh sieve.[6] The smooth enamel samples were treated by citric acid (pH 4.2) for 1 minute, then brushed using the Ca_3SiO_4 paste (liquid/powders (L/P ratio) of 1.5 mL/g) by a toothbrush for 3 minutes and rinsed with running water. Finally, the treated samples were placed in human saliva for 1 day or 3 days at 36.5°C. Both solutions were refreshed every 24 hours. Etched samples were set as a control.

Saliva collecting process

Briefly, healthy human saliva was collected from the same individual at the same time of the day for 2 days. The saliva samples were centrifuged to discard any debris and sterilized by γ radiation for 24 hours and stored at 5°C prior to use.

Characteration of remineralized enamel

The morphology and surface roughness of samples were analyzed by atomic force microscopy (AFM; AFM-Digital Instruments-Nanoscope III, Santa Barbara, CA, USA). The average roughness (Ra) was tested in three different regions of size 1 μm $\times$ 1 μm. The values were expressed as mean $\pm$ standard deviation (SD) and analyzed using two-way analysis of variance (AVOVA). A P value <0.05 was considered statistically significant.

Interface bonding and structure analysis were carried out by the dual-beam FIB system (Quanta 200 3D FIB; FEI, Leeuwarden, The Netherlands), transmission electron microscopy (TEM) with JEM-2010F (JEOL, Tokyo, Japan), selected-area electron diffraction (SAED, JEOL, Tokyo, Japan), and energy dispersion x-ray spectroscopy (EDX, JEOL, Tokyo, Japan).

Results

AFM images of the enamel samples are shown in Fig. 1. After the acid attack, the demineralization of the enamel initially occurred at the rod sheath space. Following the progress of the demineralization of the rod, the acid penetrated into the underlying sound enamel via the rod sheath leading to the demineralization of the rod core, and showed a keyhole structure (Fig. 1A). At higher magnification, the dissolution crystals were mostly separated from each other, leaving wide intercrystalline spaces (Fig. 1A_1). After soaking in human saliva for 1 day, the surface image was similar to the etched image (Fig. 1B). However, trace inorganic elements in human saliva, such as calcium, phosphate, etc., and saliva protein can penetrate into the crystal space, therefore the enamel surface showed compactness (Fig. 1B_1). However, after Ca_3SiO_4 treatment and then soaking in human saliva for 1 day, the enamel surface formed a compacted remineralization layer (Fig. 1C) and the intercrystalline spaces were filled with minerals (Fig. 1C_1). After soaking for 3 days, the crystals became more compacted and the surface became smoother (Fig. 1D,D1).

The surface roughness was further tested by AFM and the results are shown in Table 1. It is notable that the acid-etched enamel surface had higher roughness values compared with the sample soaked for 1 day after etching. Then, after Ca_3SiO_4 treatment, the surface roughness significantly decreased with the prolonged soaking time.

In order to observe the interface between the mineralized layer and the enamel matrix, the etched sample with the treatment of Ca_3SiO_4 and soaking in human saliva for 1 day was sent for FIB ion milling. The interface between the mineralized layer and the enamel matrix was observed by TEM, and the results showed that a new mineralized layer had formed and the thickness was 200—250 nm. The interface showed tight bonding, no gap occurred (Fig. 2A). In order to identify the crystal lattice structures of the enamel matrix and the remineralized layer, the electron diffraction patterns of these two regions were obtained and shown in Fig. 2B. It was confirmed that the polycrystalline structure of the remineralized layer was analogous to that of the enamel in all the areas. The chemical compositions were also closed to each other with the average Ca/P molar

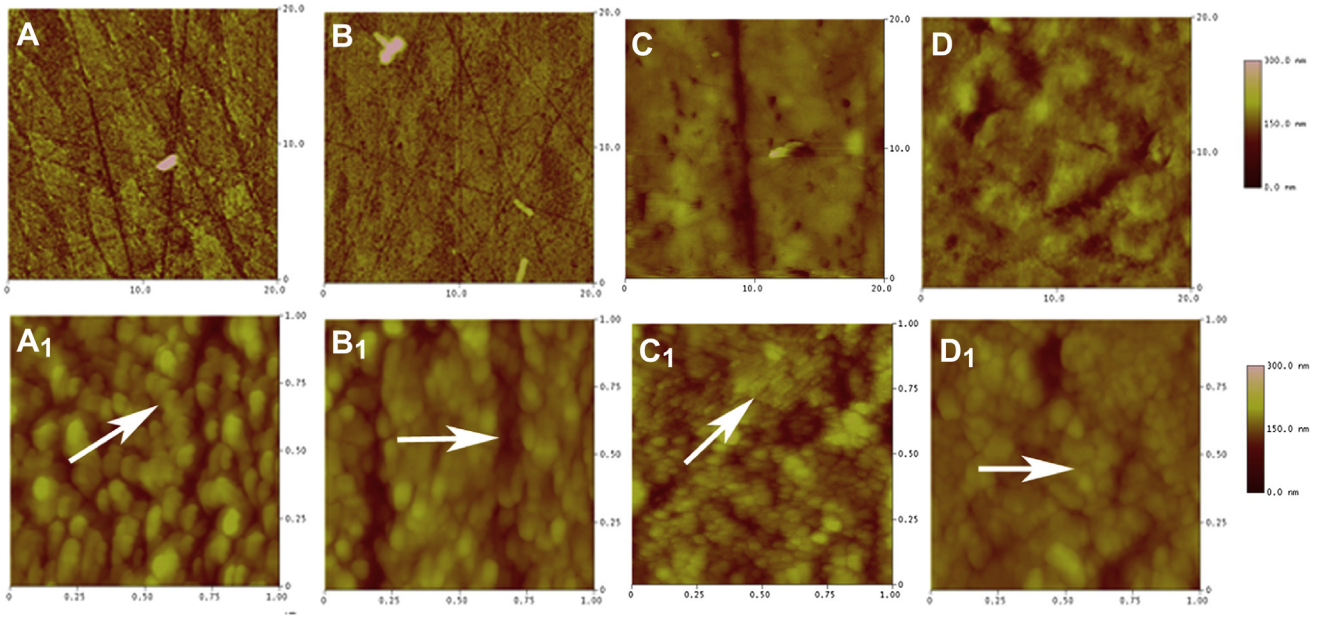

Figure 1 Atomic force microscopy (AFM) images of teeth samples. (A, A_1) Etched; (B, B_1) soaked after etching in human saliva for 1 day; (C, C_1) treated with Ca_3SiO_4 paste and then soaked in human saliva for 1 day and 3 days. Arrows indicate the hydroxyapatite (HA) crystals and intercrystalline spaces (A_1, B_1) and newly grown HA crystals (C_1, D_1).

ratios being 1:56 and 1:64, respectively (Fig. 2C). At higher magnification, the lattice parameters of these two regions presented no qualitative difference, both showing nearly hydroxyapatite plane (100) (Fig. 2D). These results showed a strongly bonding force between the mineralized layer and the enamel matrix and formation of a similar enamel crystal structure.

Discussion

Tooth enamel is the hardest tissue in the human body. Some intrinsic and extrinsic factors influence the state of the teeth, such as caries, tooth wear, and erosion. In particular, dental caries is a disease of bacterial origin. Certain bacteria can ferment sugars and other carbohydrates from the diet to produce lactic and other short chain organic acids. If the concentration of acid goes below about pH 5.5, then the enamel dissolves. Moreover, the carbonate beverages that are on the market lead to the formation of carious lesions. Based on these reasons, enamel de- and remineralization was studied. When the teeth were etched, demineralization occurred on the enamel surface. This process was more rapid in the middle of the crystals along the c axis rather than along the a- and b- axes,[7] resulting in the formation of the uniformly keyhole-like structure (Fig. 1A).[8] Although human saliva has a limited remineralization function, it is not enough to repair the etched enamel in a short period. Therefore, Ca_3SiO_4 paste was applied for repair of the etched enamel by biomineralization.

Teeth mineralization is a dynamic process, which takes place in two major pathways, "matrix-mediated" minera lization and "biologically-induced" mineralization.[9] In this paper, both mineralization pathways contribute to the growth of apatite crystals of the enamel in human oral saliva. On the one hand, bioactive Ca_3SiO_4 induces the formation of apatite onto the enamel matrix. When Ca_3SiO_4 dissolves, the protonation of Si—O groups forms rich silanols on the enamel surface and generates a more electronegative surface. These silanol groups have more triple point junctions per unit area, providing a stereochemical match for O atoms bonded to Ca^{2+} and inducing the precipitation of HA in the physiological media. Meanwhile, PO_4^{3-} ions from human saliva are attracted to Ca^{2+} through the SiO_2-rich layer and form a Ca—P mineralized layer.[10] On the other hand, saliva is the body's natural protective mechanism against decay. It contains salivary proteins, which play important roles in enamel remineralization. It is known that salivary proteins exhibit high affinity to HA. Under the effect of complicated dynamics, salivary proteins aggregate onto the enamel surface

Table 1 Surface roughness of samples.

Samples	Etched	Etched and soaked 1 d	Ca_3SiO_4 and soaked 1 d	Ca_3SiO_4 and soaked 3 d
Ra	16.74 ± 0.31	14.35 ± 0.27*	9.79 ± 0.14*	2.95 ± 0.07*

*Significant difference from the etched samples, $P < 0.05$.
Ra = average roughness.

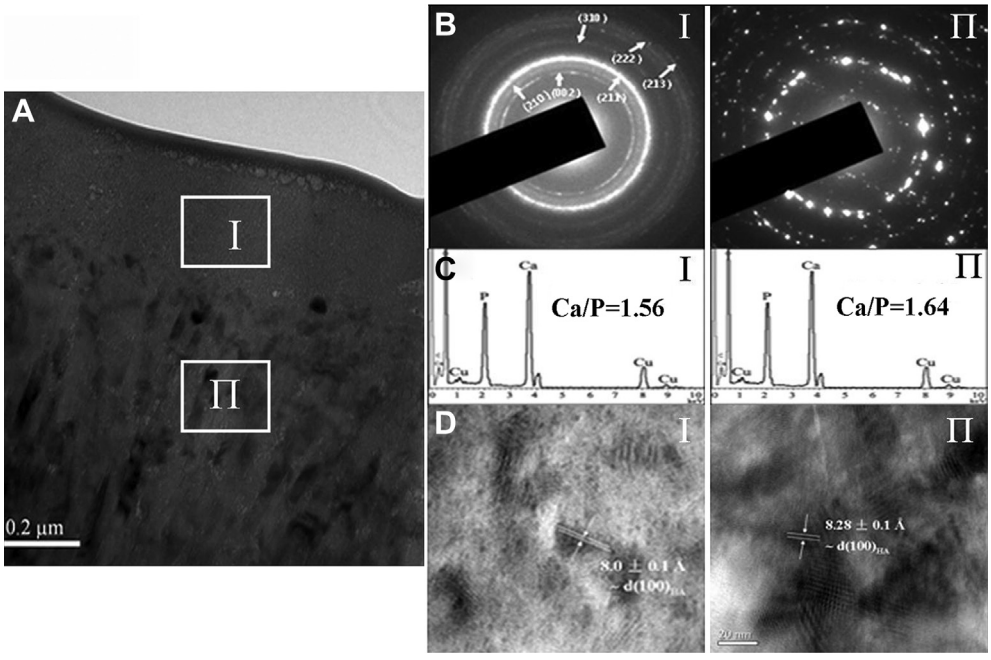

Figure 2 Micrographs of the interface between the enamel matrix and the remineralized layer. (A) Samples soaked in human saliva for 1 day. Regions I and II indicate the remineralized layer and the enamel matrix, respectively. The corresponding (B) selected-area electron diffraction (SAED), (C) energy dispersion x-ray spectroscopy (EDX), and (D) high-magnification images of regions I and II of (A). Arrows indicated the presence of the hydroxyapatite (HA) phase in the remineralized layer (B).

due to electrostatic interactions and other forces, such as Van der Waals interactions and hydrophobic interactions; salivary proteins are capable of exchange reactions with the phosphate ions of the enamel. Thus, salivary proteins will be primarily adsorbed at the enamel surface, whereas the fracture toughness of the mineralized layer can be influenced by the interaction between oral saliva and the crystal surface. Of course it is very unlikely that only ionic interactions determine the stage of enamel remineralization; a specific set of biological macromolecules of human saliva, including mucoprotein, urea, and all kinds of enzymes, are also involved in the process to improve the physical chemistry of crystal nucleation and growth and accelerate the precipitation of apatite. Although the detailed mechanism of enamel formation remains undisclosed, in this study, a compacted mineralized layer was closely bound to the enamel matrix after treatment with Ca_3SiO_4, which prevented further mineral loss. Moreover, because the surface roughness was improved, the smooth remineralized surface was better able to resist bacterial adhesion and plaque formation in the oral microenvironment.

Our findings provide important information on the role of human saliva in the remineralization of etched enamel using Ca_3SiO_4. The organic components of saliva maintained not only the integrity but also the good bonding force of the interface between the enamel and the remineralized layer. These data contribute to a better understanding of the relationship between the structural, compositional, and functional properties of dental tissue and the remineralized layer, and will further help guide the use of Ca_3SiO_4 for enamel remineralization in dental repair.

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References

1. Jensdottir T, Bardow A, Holbrook P. Properties and modification of soft drinks in relation to their erosive potential *in vitro*. *J Dent* 2005;33:569—75.
2. Pietak AM, Roel JW, Stott MJ, Sayer M. Silicon substitution in the calcium phosphate bioceramics. *Biomaterials* 2007;28:4023—32.
3. Zhao WY, Wang JY, Zhai WY, Wang Z, Chang J. The self-setting properties and *in vitro* bioactivity of tricalcium silicate. *Biomaterials* 2005;26:6113—21.
4. Laurent P, Camps J, De Méo M, Déjou Ja, About I. Induction of specific cell responses to a Ca_3SiO_5-based posterior restorative material. *Dent Mater* 2008;24:1486—94.
5. Dong ZH, Chang J, Deng Y, Joiner A. *In vitro* remineralization of acid-etched human enamel with Ca_3SiO_4. *Appl Surf Sci* 2010; 256:2388—91.
6. Zhao WY, Chang J. Sol-gel synthesis and in vitro bioactivity of tricalcium silicate powders. *Mater Lett* 2004;58:2350—3.
7. Yanagisawa T, Miake Y. High-resolution electron microscopy of enamel-crystal demineralization and remineralization in carious lesions. *J Electron Microsc* 2003;52:605—13.
8. Xue J, Li W, Swain MV. *In vitro* demineralization of human enamel natural and abraded surfaces: a micromechanical and SEM investigation. *J Dent* 2009;37:264—72.
9. Veis A. A window on biomineralization. *Science* 2005;307: 1419—20.
10. Sahai N, Anseau M. Cyclic silicate active site and stereochemical match for apatite nucleation on pseudowollastonite bioceramic—bone interfaces. *Biomaterials* 2005;26:5763—70.